CORNEA ORGAN CULTURE, ENDOTHELIUM

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INFLUENCE OF DONOR AND STORAGE FACTORS ON QUALITY OF CORNEAS STORED BY ORGAN CULTURE

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Logistic regression methods were used to analyse the influence of donor and storage factors on the quality of corneas stored by organ culture in the Bristol eye bank in 1991. Average donor age was 57 years (sd 22, n=2770) and time from death to enucleation was 8 hours (sd 7, n=2762). Corneas were placed into organ culture within 27 hours (sd 10, n+2550) of donor death and were stored for 22 days (sd 7, n=2716). Preliminary results showed that 126 of 2744 corneas (4.5%) were discarded through contamination. The risk of contamination increased with increasing death to enucleation time ($p<0.037$), but corneas from CVA donors were less likely to be contaminated ($p<0.003$). Overall, 27% of corneas were not suitable for PKP because of endothelial defects. Corneas stored for more than 4 weeks or corneas from donors over 80 years old were less likely to be suitable for PKP (both $p<0.0001$). But the likelihood of corneas having high endothelial cell densities (>2500 cells/mm^2) was reduced with donors over 20 years old ($p<0.001$), with storage times more than 2 weeks ($p<0.004$), or when corneas came from donors that died of cancer ($p<0.04$) or respiratory disease ($p<0.020$). Corneas from CVA donors, however, were more likely to be suitable for PKP ($P<0.005$). Analyses such as this are important for monitoring donor procurement practices and the efficacy of eye banking techniques.

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CORRELATION OF BIOCHEMICAL AND MORPHOLOGICAL CHANGES OF THE HUMAN DONOR CORNEA AFTER DESWELLING IN ORGAN-CULTURE

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Purpose: In histochemical investigations an adaptation of the human cornea to the dextran containing medium could be observed. Nevertheless severe alterations caused by the up-take of dextran were found. Therefore it was the purpose of this study to correlate these findings to further investigations of the energy recruitment and cellularity of the human cornea.

Material and Methods: We investigated 94 fresh human corneas and 87 corneas stored in modified MEM-medium for 28 days followed by a period of 1 to 7 days in medium containing dextran. Beside the measurement of ATP and ADP levels, the cellular densities in the corneal stroma and in the endothelial cell-layer were determined.

Results: Concerning keratocyte density a slow decrease could be observed during deswelling. Comparable results could be determined for the endothelial cell density. Regarding the biochemical parameters a steep decrease of ATP and ADP levels during the first three days in medium containing dextran was found. The latter is followed by an increase on the fourth day. Up to day seven in medium containing dextran a slight decrease could be observed.

Conclusions: Our results for ATP and ADP levels confirm the previously described phenomenon of adaptation during deswelling in medium containing dextran. In spite of that, keratocyte and endothelial cell-densities do not correlate to the biochemical and histochemical findings. Although a certain cell-loss occurs the remaining population of keratocytes is obviously able to adapt to medium containing dextran after a certain period. For the evaluation of donor corneae prior to transplantation it has to be pointed out that the endothelium alone does not reflect the viability of the whole cornea. Regarding all these findings we suggest that corneae are best transplanted on the first or on the fourth day in medium containing dextran.

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BASIC FIBROBLAST GROWTH FACTOR (bFGF) IN CORNEAL STORAGE MEDIA PROTECTS ENDOTHELIAL CELLS FROM DAMAGE CAUSED BY TOXIC AGENTS

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Purpose: We have previously shown that bFGF added to corneal storage media is able to prolong endothelial viability and thus, storage time of the cornea (IOVS 1993). In the present study, we investigated whether the presence of bFGF in the medium could protect the endothelium from damage of toxic agents. Methods: Fresh bovine corneas were stored in a modified Dexsol medium at 4°C for 14 days. Recombinant human bFGF at 20 ng/ml was added at Day 1 and D7. At D7, the corneas were additionally incubated with various concentrations (10-1000nM) of the ribosome-inactivating protein *Saporin* (gift from Dr. Lappi, La Jolla, CA). Corneas stored in bFGF-supplemented as well as in unsupplemented media served as controls. Quantitation of endothelial damage at D8 to D14 (every other day) was performed using the Janus green assay. Light and scanning electron microscopy was performed at D14. Results: Mean endothelial damage in control corneas increased from 4.2±2.0% (fresh corneas) to 18.2±6.4% after 14 days of storage. bFGF significantly reduced the overall endothelial damage to a mean value of 12.7±4.2% ($p<0.01$). When 100nM Saporin were added to unsupplemented media, endothelial damage increased to mean levels of 16.2%, 28.4% and 36.5% at days 8, 10 and 14, respectively. The same concentration of Saporin did not cause any significant additional increase in cell damage in bFGF supplemented media compared to bFGF-media without Saporin. A 3 times higher Saporin concentration was necessary to induce the same rate of cell damage in the bFGF-added group ($p<0.001$). Morphological analysis of the cell layers by light and electron microscopy confirmed the positive effect of the growth factor on endothelial protection. Conclusion: These results demonstrate the efficiency of bFGF to protect corneal endothelial cell survival against toxic agents. Our study appears interesting in view of the attempt to ameliorate the quality of the endothelium after short and medium term storage prior to corneal transplantation.

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TITLE: SENESCENCE OF CORNEAL ENDOTHELIAL CELLS

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Purpose To determine the relationship of senescence in human corneal endothelial cells (HCEC) to that reported for other cell types and to establish the effects of *in vitro* ageing on enzymes specific for the corneal endothelium *in vivo*.

Methods HCEC were passaged until they entered a non-dividing state, then assayed for viability (by MTT Tetrazolium/Thiazolyl Blue reduction) and the potential ability to replicate DNA ,by detection of Proliferating Cell Nuclear Antigen (PCNA). γ-Glutamyltransferase (GGT) & Na^+/K^+ ATPase activities were assessed by classical enzyme histochemistry. The presence of lipofuscin was determined by diastase-Periodic Acid Schiff's (PAS) staining.

Results After 3 weeks in a non-dividing state all HCEC were capable of reducing MTT. The cells showed positive reactivity for PCNA but had no detectable replication complexes. High levels of GGT and ATPase activity (relative to fibroblast controls) were detected. No lipofuscin was detected by diastase-PAS staining.

Conclusions Non dividing HCEC show clear similarities to "senescent" cells derived from different lineages. The cells retain corneal specific enzyme expression at high levels. This, together with the absence of lipofuscin, argues against growth arrest due to overt oxidative damage.